REMARKS

I. Status of the Claims

Claims 1-20, 22-24, and 26 are cancelled.

Claim 21 is amended to clarify that not all splice variants of *IG20* are to be knocked down, and to provide information on MADD and DENN-SV requested by the examiner.

Claims 21, 25 and 27 are under consideration.

II. Amendment of Claim 21 to Include Identification of MADD and DENN/SV

The structure of splice variants MADD (MADD/DENN) and DENN-SV as in claim 21, were identified in Al-Zoubi et al. (2001) as explained in the history provided by Dr. Prabhakar at the Interview of August 31, 2010, and as in the prosecution record. Further identification of these variants appear in par [0089] Example 1 of the specification, notably

MADD (acc# U77352 and U44953, 5844 bps.

DENN-SV (acc# AF440103), 5689 bps.

(see also [0108], FIGS. 2, 20)

III. Support for Amendment "Wherein Not all Isoforms of *IG20* are Knocked Down."

The examiner questioned support for the term of amended claim 21, "wherein not all isoforms of *IG20* are knocked down." This limitation is supported by the observation that *IG20* is essential for survival in normal cells so cannot be completely knocked out (see e.g. [0019]) of the specification) An aspect of the present invention is to increase cancer cell death by use of siRNAs directed toward specific isoforms, while preserving normal cells of the patient, (see [0032], [0093] of the specification for different responses of cancerous and normal cells to binding of siRNAs to *IG20* isoforms).

The inventors determined that since MADD and DENN-SV are isoforms of *IG20* required for tumor cells, and KIAA is required for normal cells, specific targeting with siRNA can attack tumor cells and not normal cells.

IV. Interview Summary

Applicant's representative, Alice O. Martin; Inventor Bellur S. Prabhakar; Jeffrey C. Norgle, University of Illinois (Applicant); Examiner Catherine Hibbert and Supervisor Christopher Low, participated in a telephone interview on August 31, 2010.

At the interview, the following issues were discussed:

Rejection of Claims 21, 25 and 27 over 35 U.S.C. §103.

In the Office Action mailed July 8, 2010, page 5, the Examiner rejected the claims as obvious:

> Absent evidence to the contrary, one would have a reasonable expectation of success combining the teachings of the art because the use of the siRNA technology for abrogation of specific gene expression in substitution for antisense technology was routinely practiced at the time the invention.

Office Action, page 5

The art cited was Al-Zoubi et al. or Efinova; Lim and Cho; and Thompson.

In the interview of August 31, 2010, to clarify why there was no "reasonable expectation of success..." based on these publications, Dr. Prabhakar gave a summary of the history leading to the present invention, focusing on evolution of terminology, to clarify why the combination of publications cited do not make the present claims obvious.

HISTORY

Chow 1996 - did not report satisfactory, operative splice variants as in the present claims.

Chow VT et al. (DNA Seq 1996;6:263-73) reported cloning of DENN (Differentially Expressed in Normal and Neoplastic tissues) and a variant called DENN-SV (Genome 1998;41:543-552). These cDNAs were cloned by subtractive hybridization of normal and neoplastic tissues.

Schievella 1997 - MADD cloned
MADD = DENN (Sequences)

Shortly after that, **Schievella** AR et al (J Biol Chem 1997;272:12069-75) reported cloning and initial characterization of a cDNA they termed MADD (Mapkinase Activating Death Domain containing protein) because it was isolated using the cytplasmic domain of the TNFR1 in a yeast-two-hybrid system. The intent of the cloning was to isolate proteins involved in inflammation mediated through TNFR1. Sequence comparison revealed that MADD and DENN are identical. Currently, the official Genbank designation for the gene is MADD.

Prabhakar et al. - in the 1980's first addressed *IG20*; cloned, 4 splice variants.

Subsequently, **Dr. Prabhakar** cloned *IG20* (Insulinoma-Glucoganoma clone 20). The cDNA was cloned through subtractive hybridization of a human pure Insulinoma single strand cDNA library with a pure human Glucagonoma single strand library (Al-Zoubi AM et al J Biol Chem 2001; 276:47202-11). A careful analysis of the cDNA sequence and RT-PCR using different primers and mRNA from different tissues revealed that *IG20* gene can encode 4 different splice variants namely, *IG20*pa, MADD/DENN, *IG20*-SV2 and DENN-SV. These SVs resulted from alternative splicing of long exon 13 (13L) and exon 16. *IG20*pa contains both, while MADD and *IG20*-SV2 lack exon 16 and 13L respectively, and DENN-SV lacks both (Al-Zoubi AM et al J Biol Chem 2001; 276:47202-11).

Lim 2002, 2004
ODNs Target DENN, apoptosis cancer cells
(Didn't take *IG20* splice variants into account)
(ODNs COULD ALSO KNOCK DOWN ALL ISOFORMS OF *IG20*)

Lim KM et al (2002). Mol. Carcinog. 35: 110-126; and Lim KM (2004). Int J Cancer 109: 24-37 showed that ODNs that can target DENN can result in apoptosis of cancer cells. At that time they did not take into account the existence of various *IG20* splice variants that Dr. Prabhakar had identified. Subsequently, it became apparent that ODNs used to knock-down MADD/DENN could also knockdown all isoforms of the *IG20* gene (Gen Bank designation: MADD) namely *IG20*pa, MADD, IG20-SV2 and DENN-SV. [Please note that since the sequences of two of the splice variants Dr. Prabhakar identified were identical to that of originally reported MADD and DENN-SV sequences, that terminology was retained.]

Wada 1997 - Mouse Ortholog of *IG20* Gene-Knockout = Death

Wada, M, et al. [*(*1997*)* J. Biol. Chem. 272, 3875-3878] had cloned rat Rab3a-GEP and knockouts of this gene in mice resulted in the death of the animal shortly after birth [Yamaguchi K et al., Proc Nat Acad Sci USA 2002;99:14536-41; and Tanaka M, et al. Moc Biol. Cell. 2001;12:1421-30] indicating the critical function of the gene in neurotransmission. It turned out that this gene is the mouse ortholog of the *IG20* gene.

Villar 2004 - ODNs (Same as **Lims**) Knockdown MADD/DENN, i*n vitro* neuronal cell apoptosis.

In a subsequent study **Villar** (2004). [Proc. Nat. Acad. Sci. USA 101:4210-4215] showed that knockdown of MADD/DENN using ODNs can lead to neuronal cell apoptosis in vitro. The ODNs used in this study were the same as those used by Lim KM et al [(2002). Mol. Carcinog. 35: 110-126; and Lim KM (2004)]. Here again the ODNs used could knockdown all isoforms of the *IG20* gene.

Prabhakar et al. 2008 Found MADD Critical for cancer cell survival

2 more isoforms found in nervous system (splice variants) of *IG20* are *IG20*-SV4 and KIAA. Loss of KIAA (*IG20*SV4) = Death

Subsequently, Dr. **Prabhakar** developed "isoform specific" siRNAs to selectively knockdown one or more of the splice variants to understand their relative importance in cancer. Additionally, he discovered that not all cancer cells expressed all 4 isoforms and some expressed only the MADD and the DENN-SV isoforms. Using a combination of cells that expressed all 4 isoforms or only the MADD and DENN-SV isoforms, and different sIRNAs that specifically targeted exon 13L (to knockdown *IG20*pa and MADD), exon 16 (to knockdown *IG20*pa and IG20-SV2) and mid-siRNA targeting exon 15 (exon 15 is expressed in all isoforms of the *IG20* gene and like the previously used ODNs it can knockdown all isoforms). More importantly, Dr. Prabhakar constructed cDNAs with third base substitutions in the mid-siRNA targeted region so that they can be expressed in a cell in which all endogenous isoforms were knocked down using the mid-siRNA (that targets exon 15).

Although both 13L-siRNA and 16-siRNA could knockdown *IG20*pa, only the 13L-siRNA could cause cancer cell apoptosis. This indicated for the first time that specifically MADD might be critical for cancer cell survival. Using cancer cells that express only MADD and DENN-SV Dr. Prabhakar was able to selectively knockdown MADD using 13L-siRNA and showed that MADD was required for cancer cell survival. Moreover, he was able to rescue cells from undergoing apoptosis only upon re-expression of mid-siRNA resistance MADD, and not any of the other isoforms in the absence of endogenous isoforms (endogenous isoforms were knocked down using Mid-siRNA). (Mulherkar N et al., Oncogene 2006; 25:6252-61.)

At this juncture, Dr. Prabhakar had very compelling evidence that showed a critical role for MADD in cancer cell survival. However, his group was puzzled by the publications in which knock down of "MADD/DENN" had been shown to result in neuronal cell death, while RAb3a-GEp knockout could result in the death of the animal. Therefore, they further investigated the role of *IG20* splice variants in neuronal cells [(Li LC, et al, (2008) Cancer Res, 68:7352-61)]. Surprisingly, his group identified two additional isoforms (splice variants) of *IG20* namely, *IG20*-SV4 and KIAA that are uniquely expressed in certain cells of the nervous system and none of the non-neuronal cells or tissues tested to date. This confirmed an earlier report from Coppola T, et al (2002. Biochem J. 362:273-9) that showed that the KIAA isoform contains the Rab3a-GEP activity required for neuronal transmission. Collectively, these results indicated that it is the loss of KIAA function that results in neuronal cell death in vitro upon MADD/DENN knockdown and death in Rab3aGEP knockout in mice.

Use of siRNAs to selectively knockdown *IG20* isoforms is not in the prior art. Lim et al. and Del Villar et al. have shown the potential of knocking down "DENN" using ODNs. What neither group appreciated at that time is that the ODNs they used could target all known isoforms of the *IG20* gene including KIAA and the resultant harmful effects including death of the animal. Therefore, none of the earlier papers cited in the response revealed the full complexity of *IG20* gene expression, selective expression of different isoforms in different tissues, unique functional attributes of each of the splice variants that Dr. Prabhakar et al. have so clearly defined, their relative importance for normal neuronal

function and survival of the animal (KIAA), cancer cell survival (MADD) and proliferation (DENN-SV). Therefore, it is the discovery of various isoforms, their functional characterization and the ability to identify specific siRNAs that can selectively knockdown various isoforms that allowed the inventors to develop cancer therapeutics without the unintended potential negative consequences, including the death of the animal, associated with the use of ODNs.

In addition to discussing why the history should make clear the invention is not obvious, claim amendments to reflect the history were promised.

V. A Prima Facie Case of Obviousness is Not Established

"New Grounds" of rejection under 35 U.S.C. §103(a) over the following combination of publications were in the Final Rejection, and maintained in part in the Advisory Action.

Claims 21, 25 and 27 are not obvious based on any combination of Al-Zoubi et al. or Efinova, et al.; Lim and Chow; and Thompson.

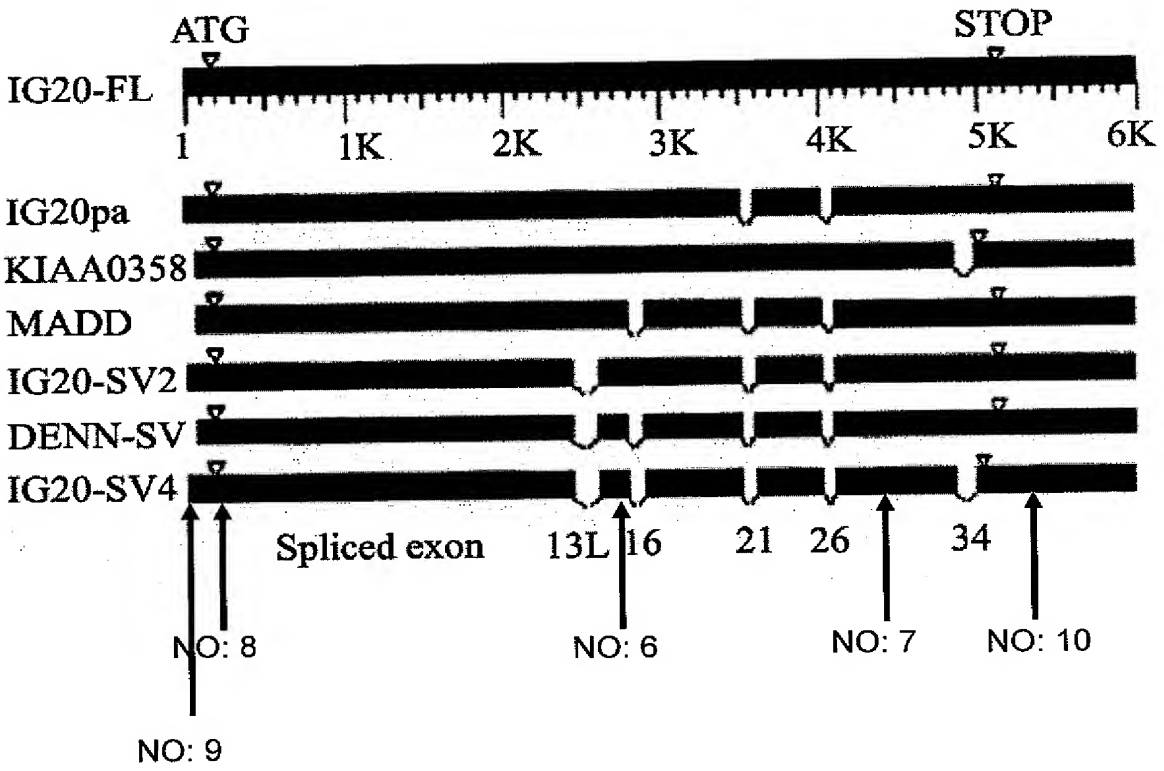
As explained above in IV, and reiterated by the examiner, the inventors discovered and claimed:

> the specificity (i.e. the sequence of the genes being targeted) of the human splice variants of IG20 that are targeted by siRNA is crucial for the invention to work in animal models because some siRNAs and ODNs that could target human splice variants of IG20 in the cell could end up knocking down some or all of the unintended splice variants and thus could result in the death of the animal model.

Interview Summary, September 16, 2010.

The following table illustrates how a variety of ODNs, whose sequences have been previously published, can target all isoforms of the *IG20* gene identified to date. Please note that different siRNAs can target different exons (i.e. numbered 6-9) or non-coding regions (i.e. numbered 9 and 10). Because those exons are expressed in all isoforms, treatment with a particular siRNA in the publications cited results in knock down of all isoforms.

The published AS sequence of *IG20*/MADD and their targeting positions previously are



(Seq. No.6) **5'-CCAGTCTCAAGCTGTTGGGCC-3'** can target exon 15 region

(Seq. No.7) **5'-TGTAGGAGATGAGGTTGTG-3'** can target exon 28 region

(Seq. No.8) **5'-CATGGTTCCAATTCATCAGT-3'** can target exon 2 region

(Seq. No.9) **5'- GAAGCATCAGGGCACCAA-3'** can target 5'-UTR

(Seq. No.10) **5-ATAACGCCAGGGACAAGGACA-3** can target 3'-UTR

The examiner questioned support for the term of amended claim 21, "wherein not all isoforms of *IG20* are knocked down." This limitation is supported by the observation that *IG20* is essential for survival in normal cells (see e.g. [0019]) of the specification) and an aspect of the present invention is to increase cancer cell death by use of siRNAs directed toward specific

isoforms, while preserving normal cells of the patient, (see [0032], [0093] of the specification for different responses of cancerous and normal cells to binding of siRNAs to Ig20 isoforms).

Unlike the above mentioned ODN sequences, the sequences that the present inventors have identified and claimed in the present application, bind to particular exons that are differentially expressed in different isoforms. Therefore, knockdown using a specific siRNA will knockdown only those isoforms in which that particular targeted exon is expressed. This allows for selective knockdown of the desired isoforms and thus reduces the unintended negative effects and are not lethal.

The anti-sense sequences reported by others are shown below.

Summary of Published AS sequence target position in IG20/MADD

Sequence serial #	Sequence	Target exon	Reference
Seq. No.1	5'-TCACTTGCCAGTCTCAAGCTG-3'	Exon 14	US Pat. Pub. # 20030162734 published sequence
Seq. No.2	5'-CCAGTCTCAAGCTGTTGGGCC-3'	Exon 14	US Pat. Pub. # 20030162734 published sequence
Seq. No.3	5'-GAACTTCTTCTTTTGCACCAT-3'	Exon 2	US Pat. Pub. # 20030162734 published sequence
Seq. No.4	5'-TCCAAGGGACAGGTACCTGTC-3'	Exon 27-28	US Pat. Pub. # 20030162734 published sequence
Seq. No.5	5'-GCTAGAGACAGGCCGGGGCGG-3'	Exon 36	US Pat. Pub. # 20030162734 published sequence
Seq. No.6	5'-CCAGTCTCAAGCTGTTGGGCC-3'	Exon 15	Del Villar and Miller 2004
Seq. No.7	5'-TGTAGGAGATGAGGTTGTG-3'	Exon 28	Del Villar and Miller 2004, Lim KM., et al. 2002, Lim KM., et al. 2004,
Seq. NO.8	5'-CATGGTTCCAATTCATCAGT-3'	Exon 2	Lim KM., et al. 2002, Lim KM., et al. 2004,
Seq. NO.9	5'- GAAGCATCAGGGCACCAA-3'	5' UTR	Lim KM., et al. 2002, Lim KM., et al. 2004,
Seq. NO.10	5-ATAACGCCAGGGACAAGGACA-3	3' UTR	Lim KM., et al. 2002, Lim KM., et al. 2004,

A determination of obviousness requires that "the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved." *KSR International Co. v. Teleflex, Inc.*, -- U.S. --, 127 S.Ct. 1727, 1734, 82 U.S.P.Q.2d 1385 (2007) *quoting Graham v. John Deer Co.*, 383 U.S. 1, 17 (1966). In making a determination of obviousness by looking at the teachings of multiple patents, one should consider

> the effects of demands known to the design community or present in the market place; and the background knowledge possessed by a person having ordinary skill in the art, all in order to determine whether there was an apparent reason to combine the known elements in the fashion claimed

> by the patent at issue. **To facilitate review, this analysis should be made explicit.**

KSR, 127 S.Ct. at 1740-41 (*emphasis added*). "[A] patent composed of several elements is not proved obvious merely by demonstrating the each of its elements was, independently, known in the prior art." *Id.* at 1741.

Clearly the present claims were unpredictable from the publications cited by the examiner, because no one before the present inventors taught use of specific siRNAs for isoforms, and that not all should be applied at the same time.

Dr. Prabhakar has explained why the publications cited to support an obviousness rejection could not be combined and produce an operative invention, as claimed herein. Therefore, please allow pending claims.

IV. Conclusion and Summary

No fees are believed due at this time, however, please charge any deficiencies or credit any overpayments to deposit account number 12-0913 with reference to our attorney docket number (21726-103049).

Respectfully submitted,

Date: January 6, 2011

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